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Air Pollutants and the Facilitation of Cancer Metastasis

by A. Richters* and K. Kuraitis*

Studies have been carried out to determine whether the inhalation of ambient levels of nitrogen dioxide (NO_2), a common air pollutant, could influence the frequency of blood-borne cancer cell metastasis to the lungs. B16 mouse melanoma cells were used as an *in vivo* test model. The results have indicated that animals inhaling ambient levels of NO_2 developed a significantly higher number of melanoma nodules in their lungs than the animals inhaling filtered air. Thus, a new concept for the action of air pollutants is proposed. The question is raised whether similar events are taking place in urban human populations.

The presence of pollutants in the environment, especially those with carcinogenic properties, has been of great concern to environmental health scientists. In view of this, many studies have been directed toward the identification of cancer-causing agents in the environment (*1-4*). However, the problem of cancer involves not only the development and presence of neoplastic cells at a primary site, but also the ability of these cells to migrate, seed, and proliferate in distant organs and tissues. The importance of cancer cell dissemination and metastasis has been emphasized by many investigators and has been stated particularly well by Day, who wrote that, "even though the cause of cancer is important, in the clinical case it is the spread—the phenomenon of metastasis—that is of much more immediate concern in the human situation" (*5*). Moreover, considering that a significant segment of the population is already affected by cancer together with the probability that one in four individuals will develop cancer (*6*), the question arises as to the role environmental pollutants play not only in the causation of cancer or carcinogenesis, but in the progression of the disease, particularly the dissemination of cancer cells and development of metastases.

The development and progression of cancer is a very complex process (Fig. 1). It is possible that different air pollutants could act at different sites in the sequence of cancer causation and progression. In general, one could say that certain air pollutants could participate in the process of carcinogenesis and others in the process of cancer cell dissemination and metastasis (Fig. 2). With respect to carcinogenesis, noxious air pollutants could act as initiators or complete carcinogens, cocarcinogens or promoters leading to the development of cancer. In the case of cancer cell dissemination and metastasis, certain air pollutants could act as facilitators by exerting their effects on the host in a noncarcinogenic manner. Thus, individuals with existing cancer or potential cancer patients should be at highest risk since it is recognized that most cancer patients have circulating cancer cells (*7, 8*) and in some instances cancer cells have even been demonstrated in the circulation of individuals without clinical signs of cancer (*9, 10*). In addition, circulating cancer cells are also found in peripheral blood of tumor-bearing animals (*11*), indicating the universality of this phenomenon. Moreover, there are several known conditions which may favor the development of cancer cell metastases from such circulating cells. The best documented of these conditions are: immune suppression, endothelial cell alterations, cancer cell homotypic or heterotypic aggregation, cancer cell interactions with components of blood clotting system and tissue damage in general (*12-17*). Several of the mentioned conditions occur as a result of nitrogen dioxide (NO_2) inhalation (*18-23*), and thus one may expect that air pollutant inhalation could facilitate or en-

*Department of Pathology, University of Southern California School of Medicine, Los Angeles, CA 90033.

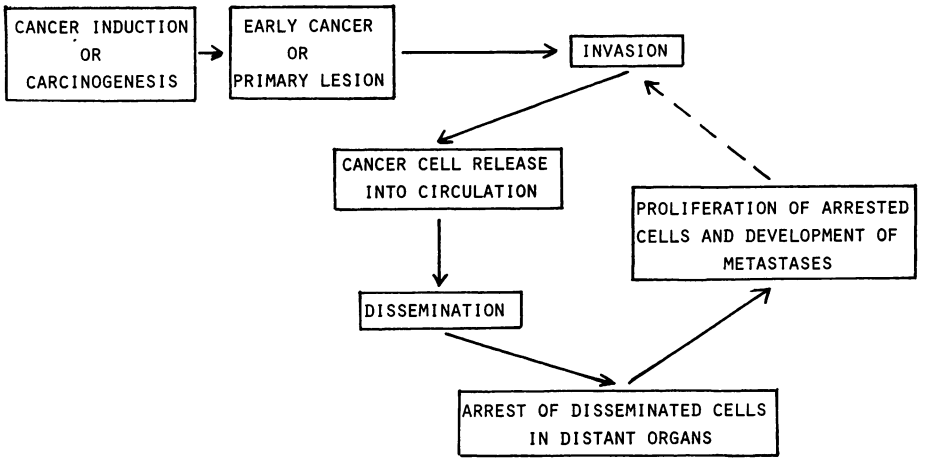

FIGURE 1. The course of cancer. A simplified outline of cancer course. Note that metastases can also invade and enhance the progression of the destructive process.

hance circulating cancer cell metastasis. The lung in particular is a likely candidate for such metastases development since it is a common site for metastasis in general and is affected by the inhalation of air pollutants as well. Recent experiments in our laboratory with a mouse melanoma model have indeed demonstrated that inhalation of ambient levels of NO_2 facilitates blood-borne cancer cell metastasis to the lungs (*24-26*) and the animals die if the metastatic nodules are permitted to progress. The number of melanoma nodules developing in the lungs was significantly higher ($p < 0.005$) in the exposed animals.

In this article we express a concern for human health and we present additional data from ambient level NO_2 and from ambient urban air inhalation experiments (Table 1). Most importantly, we are introducing a new concept about the possible action of air pollutants (Fig. 2). More specifically, the common air pollutant (NO_2) is implicated in the facilitation of blood-borne cancer cell dissemination and lung metastasis development. It should be mentioned that inhalation of cigarette smoke also has been linked to the enhancement of cancer cell metastasis by other investigators employing a different experimental system (*27*).

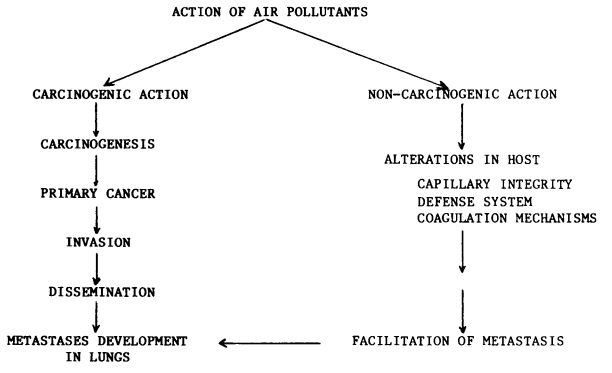

FIGURE 2. Action of air pollutants on the process of carcinogenesis and the facilitation of cancer cell metastasis. If an air pollutant acts as facilitator, it affects the host in non-carcinogenic manner.

The details of our experimental methodologies have been described before (*25, 26*). In brief, the animals (C57 BL/J6 mice) were divided into three equal groups and were designated as NO_2-exposed group, filtered air control group and ambient air (vivarium room air) group. The NO_2 exposed group and the filtered air controls were housed in identical environmental chambers while the ambient air group was housed in regular vivarium room environment. The prescribed NO_2 concentration was delivered to the NO_2 environmental chamber by a method described previously (*28*) which has been in use in this laboratory for several years. The NO_2 levels in environmental chambers and in room air were monitored by the Saltzman method (*29*) and a chemiluminescence NO_x detector. The animals were exposed to NO_2 7 hr/day, 5 days/week for a designated number of weeks. After the designated exposure period the three groups of animals were infused with 10^5 B16 F10R1 melanoma cells via the lateral tail vein and were housed in vivarium room air for an additional 3 weeks. Following this time period animals were killed, lungs were removed en bloc, inflated with 10% acetate-buffered formalin, and the pigmented melanoma nodules were counted by stereomicroscope.

Table 1. Frequency of pulmonary metastases.

Duration of exposure, weeks	Treatment	No. of animals	Mean number of nodules per lung	p-value[a]
8	Filtered air	29	10.7	FA vs. NO_2 NS
	NO_2, 0.5 ppm	23	9.3	
	Ambient air	26	10.7	AA vs. NO_2 NS
12	Filtered air	29	10.1	FA vs. NO_2 $p = 0.05$
	NO_2, 0.3 ppm	25	15.6	
	ambient air	28	15.1	FA vs. AA $p = 0.03$
12	Filtered air	48	35.0	FA vs. NO_2 $p < 0.01$
	NO_2; 0.4 ppm	51	50.0	

[a]Mann-Whitney-Wilcoxon test. FA = filtered air; AA = ambient air; NS = not significant.

We have used the foregoing experimental procedures to test the effects of 0.8, 0.5, 0.4 and 0.3 ppm of NO_2. The 0.8, 0.4, and 0.3 ppm NO_2 exposures have facilitated formation of metastases in the lungs of exposed animals. The 0.5 ppm NO_2 exposure has not shown facilitation. It should be pointed out that the length of NO_2 exposure in the latter experiment was 8 weeks instead of 10 or 12 weeks employed in our other experiments. It is of interest that 8-week exposures to ambient vivarium air also showed the lack of facilitation (Table 1). We interpret these observations as being indicative of an exposure length–response relationship, not necessarily a dose relationship. Namely, prolonged exposures to low level ambient air pollutants or low level NO_2 may be more detrimental to the host than exposures of a shorter duration to slightly higher ambient levels of pollutants.

The facilitation of metastasis development by inhalation of ambient vivarium room air is more difficult to relate to a specific air pollutant since the composition of room air could be very complex. The latter condition of course resembles closer the daily human exposures in Los Angeles or other urban areas. With respect to NO_2 levels in vivarium room air during the experimental period, the monitoring showed NO_2 levels below 0.1 ppm. In addition, experiments have been carried out in two different buildings in the same general Los Angeles area and revealed similar NO_2 levels, eliminating the possibility that a particular room or a building may play a role. The NO_2 level in the ambient outside air during the experiment period has fluctuated between 0.01 and 0.2 ppm.

The mechanism involved is not clear, but it is possible that several of the aforementioned conditions that affect the host and favor the development of cancer metastasis may be involved. Some of our own data generated from studies pertaining to the spleen may be relevant to the mechanisms involved, since we have observed different spleen responses in the early and late phases of NO_2 exposures (*30, 31*). The latter observations considered together with other immunological studies which suggested immune stimulation in the early phases and suppression in the late phases of NO_2 exposure (*18*) may be part of the mechanisms involved in the facilitation of metastases development.

With respect to the human experience, epidemiological studies have shown increased mortality rates from cancer in polluted urban areas (*32, 33*) and could be interpreted as being due to an increased frequency of metastasis. However, other studies have not demonstrated this correlation (*34, 35*). Most importantly, epidemiological studies designed to study specifically the frequency of metastases are missing. Several existing reports often equate the increased incidence of cancer with an increased incidence of mortality and thus present problems for appropriate interpretation. Epidemiological studies where the frequency of cancer metastases development could be correlated with a specific ambient environmental contaminant are needed urgently.

Thus, the data from our experimental studies provide the first evidence that under certain conditions inhalation of ambient levels of NO_2 or polluted urban air can facilitate blood-borne cancer cell metastasis to the lungs. Even though our data come from an experimental animal model, we consider these findings highly relevant to human health. Namely, because studies with radiation and other traumatizing treatments in cancer patients have resulted in enhancement of cancer cell metastasis and the same events have been observed with experimental animals. Thus, our

results with air pollutant inhalation and facilitation of blood-borne cancer cell metastasis in animals may well indicate what may be happening in human populations. We feel the findings are also relevant to air quality standard setting since all available evidence should be considered.

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